IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Eleftheria Maratos-Flier et al. Art Unit : 1647
Serial No. : 09/159,068 Examiner : C. Saoud
Filed : September 23, 1998
Title : REGULATION OF EATING BEHAVIOR

Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. §1.132 OF DR. ELEFTHERIA MARATOS-FLIER

I, Eleftheria Maratos-Flier, a citizen of the United States of America, residing in Newton, Massachusetts, hereby declare as follows:

1. I am currently an Investigator at Joslin Diabetes Center, Associate Professor of Medicine at Harvard Medical School and Associate Physician at Beth Israel Deaconess Medical Center. I earned my M.D. degree from Mount Sinai Medical School and completed residency training at George Washington University and Beth Israel Hospital, Boston. I am a former Mary K. Iacocca Fellow at Joslin Diabetes Center.

2. I am a co-inventor of the invention claimed in the above-identified patent application, which is being filed herewith. I have read and understand the contents of the present patent application. The present claims are directed to a method of inhibiting appetite or weight gain. The method includes administering an effective amount of an antagonist of MCH to a subject, where the antagonist binds an MCH receptor.

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

June 21, 2002
Date of Deposit

Maria Keen
Signature

Maria Keen
Typed or Printed Name of Person Signing Certificate

3. I have also been advised and understand that the Examiner has rejected the previously pending claims of the parent application. I further have been advised and understand that this rejection, in part, is based on the Examiner's assertion that:

> the specification does not provide clear guidance as to which amino acids (i.e., structural elements) of the native protein are critical to the biological activity of an antagonist and which amino acids should be altered in order to obtain an MCH antagonist.

4. It would not require undue experimentation for one skilled in the art, guided by the application, to determine what peptide analogs of MCH, or non-peptide molecules that bind an MCH receptor, would function as antagonists of MCH. As discussed in detail below, the results and models provided in the application are effective for predicting what MCH mutants which will function as antagonist. Sufficient guidance is provided for a skilled artisan to determine whether a molecule (e.g., a peptide or non-peptide-based molecule) that binds an MCH receptor has MCH antagonist activity. Furthermore, the specification teaches that MCH antagonists inhibit eating behavior, and that is precisely what other investigators have confirmed (e.g., see Takekawa et al. (2002) European J. Pharmacol. 438:129-135, discussed in detail below).

5. Contrary to the Examiner's statement, the results obtained from the skin bioassays described in the application are indeed sufficiently predictive of the structure-function relationship of MCH to determine what MCH analogs would act as antagonists. The results (and the predictions made from the results about MCH activity) have been essentially confirmed by other investigators, e.g., by Audinot et al. (2001) J. Biol. Chem. 276:13554-13562 (copy enclosed). For example, the specification provides that "modifications of residues within the ring. . . resulted in analogs with greatly reduced MCH activity. These results support the suggestion that the MCH activity is elicited from the cyclic segment (MCH 5-14) of the peptide" (see page 22, lines 31-34). Describing results testing analogs of MCH in human cells, Audinot

similarly concludes that "the cyclic part of MCH plays an essential role for activity" (Audinot at p.13557). Moreover, the specification provides as follows.

> Because fragment analogs, which are N terminal deleted, e.g., those lacking residues 1-4, are equipotent to native MCH, they appear to not be required for MCH activity. The same was concluded for residues 16-17 in the C-terminal end of the peptide" (page 21, lines34-37).

Reaching the same conclusion, Audinot et al. state that "[T]he last 2 and the first 5 amino acids of MCH... were not essential since deletions of these amino acids only decreased ~10-fold the potency to inhibit forskolin-induced intracellular cAMP level" (Audinot at p. 13558, top of first column).

6. In light of the above results showing that the ring structure is most important for MCH activity (which results are described in the specification and confirmed by Audinot et al.), the specification further predicts that antagonists may have "1, 2, 3, 4, 5 or more residues within the ring modified or substituted with a nonconserved amino acid from the table provided herein" (page 26, lines 1-6, as presently amended). Once again, this prediction is confirmed by Audinot et al., who show that eight MCH analogs that were mutated in the ring structure were antagonists (see Audinot at al. p. 13561, second column, first and second full paragraphs). In one particular case, Audinot et al. show that an analog having a ring structure "in which not less than five residues were substituted by non-natural residues" was a potent antagonist. (See Audinot at al., p. 13559, second column).

7. It is particularly noted that Audinot et al. tested only 57 MCH analogues for activity in human cells, and found at least eight (or about 14%) that were MCH antagonists. Of these eight antagonists, all were mutated in the ring structure of MCH (see Audinot at al. p. 13561, second column, first and second full paragraphs). As discussed above, the ring structure of MCH is explicitly predicted in the specification to be important for MCH activity and to be mutated in an antagonist. Furthermore, Audinot et al. were not searching for MCH antagonists in particular, but were doing "an extensive and detailed structure-activity relationships study of MCH." (See Audinot at al., p.13555, first column). This is clear evidence that only routine experimentation would be required of a skilled artisan to make and use MCH

antagonists that are peptide analogs. For example, one of ordinary skill in the art would have been able to modify the ring structure of MCH as taught by the specification, and tested the activity of the modified molecule in an assay provided in the application, e.g., an assay for synaptosome binding, which can be used to test the ability of a compound to competitively antagonize a naturally occurring MCH receptor. See, e.g., page 18, lines 5-8 of the specification.

8. In summary, the specification teaches particular regions of MCH that can be mutated to make antagonists and teaches various assays for following activity. Audinot et al., (2001) J. Biol. Chem. 276:13554-13562, published after the filing date, mutated MCH and tested the mutants for activity against a human cell transfected with a human MCH receptor. Using this assay system, Audinot et al. found numerous antagonists (8 of 57 mutant peptides made). All of the antagonists included changes in the MCH ring structure, as taught by the specification.

9. With respect to non-peptide antagonists, the specification also provides sufficient guidance to enable a skilled artisan to make and use a non-peptide antagonist of MCH that binds an MCH receptor in the claimed methods. Indeed, the fact that an MCH antagonist inhibits eating behavior has been confirmed by other investigators. Using similar methods to those described in the specification, other researchers have found a chemical compound, a (-) enantiomer of N-[6-(dimethylamino)-methyl]-5,6,7,8-tetrahydro-2-naphthalenyl]-4'-fluoro[1,1'-biphenyl]-4-carboxamide, referred to as T-226296, that selectively antagonizes the MCH receptor and suppresses food intake in an animal. See Takekawa et al. (2002) European J. Pharmacol. 438:129-135 (copy enclosed). In particular, the present specification describes several *in vitro* assays for binding to and antagonizing a naturally occurring MCH receptor and an *in vivo* assay for appetite suppressing antagonistic activity. As detailed below, a similar approach was used by Takekawa et al. to identify a non-peptide antagonist that functions to inhibit appetite in a subject.

10. For example, the specification provides as follows.

> The inventor has discovered that MCH induces eating behavior. The invention includes a number of methods for evaluating treatments or agents for MCH agonist or antagonist activity. Some methods use *in vitro* assays, while others use cells, and yet others use animals. Methods referred to herein can be used individually, or in combination, to evaluate agents for MCH agonist or antagonist activity. For example, relatively rapid *in vitro* or cell based assays can be used as an initial screen and an animal assay used as a secondary screen (page 4, lines 17-23)

An exemplary method of identifying an MCH antagonist (of several methods described in the specification) is provided at page 18, lines 5-23, which provides as follows.

> Synaptosome Binding:
> The ability of a compound to bind a naturally occurring MCH receptor, e.g., the ability of analogs to competitively antagonize MCH, can be determined utilizing radiolabeled MCH in binding assays. A competitive antagonist can prevent MCH from interacting with its receptor and less radioactivity would be bound.
> ***
> Generally, the tissue sample of interest is homogenized when preparing membranes or synaptosomes, or digested with enzymes for whole cells. Whole cells, membranes or synaptosomes can then be isolated by centrifugation. For example, membranes can be prepared by homogenizing the tissue sample, and centrifuging the resultant solution. The supernatant is collected and centrifuged. The pellet is resuspended and passed through a small gauge needle. The crude membrane pellet is resuspended in an appropriate binding buffer. Membranes are exposed to a constant concentration of radiolabeled MCH and varying concentrations of the analog of interest. Unbound ^{3}H-MCH is separated from the bound ^{3}H-MCH by a rapid filtration over fiber glass filters.

11. This assay would allow a skilled artisan to identify a peptide or non-peptide compound that prevents radiolabeled MCH from binding to a cell membrane fraction as a competitive MCH receptor antagonist. Because MCH was known to be present in brain (see "Background of the Invention"), one would expect that a synaptosome fraction, as described in the assay above, would contain a naturally occurring MCH receptor and could be used in this type of assay. In Takekawa et al., the authors used precisely this type of *in vitro* method to identify a compound that antagonizes the MCH receptor. Takekawa et al. prepared membranes from cells expressing an MCH receptor and showed that their test compound T-226296 displaced the binding of MCH to its receptor (see section 3.2 of Takekawa). As discussed above, this is

precisely the type of activity that the synaptosome binding assay described in the application would evaluate.

12. In addition, the specification also describes a two-step method, including an *in vivo* assay that can be used to confirm that a compound that binds and antagonizes the MCH receptor has appetite suppressing activity in a subject. For example, the specification provides as follows.

> In yet another aspect, the invention features a two-phase method (e.g., a method having an *in vitro* and an *in vivo* phase) for evaluating an agent, e.g., for the ability to modulate, e.g., to inhibit or promote, an interaction of an MCH peptide with a naturally occurring ligand of MCH, e.g., an MCH receptor, e.g., MC3-R. The method includes steps (i) and (ii) of the method described immediately above performed *in vitro*, and further includes: (iii) determining if the agent modulates the interaction *in vitro* and if so; (iv) administering the agent to a cell or animal; and (v) evaluating the *in vivo* effect of the agent on an interaction, e.g., inhibition, of an MCH peptide with a second polypeptide, e.g., by the effect on eating behavior. (See page 8, lines 30-38).

> rodents, e.g., rats, can be used to assay MCH activity *in vivo*. A Teflon catheter can be inserted into the third ventricle of a rat and cemented into place. MCH or its analogs, e.g., agonists or antagonists, can be introduced by way of the catheter at various concentrations, and their effect on eating behavior determined. (See page 18, lines 34-38)

In Takekawa et al., the authors confirmed the appetite suppressing activity of T-226296 using a similar rodent model, differing in the mode of administration of the antagonistic compound (see section 3.4 of Takekawa et al.).

13. In summary, using the methods such as those provided in the specification or very similar methods, other investigators have been able to identify MCH antagonists and perform the claimed methods. Takekawa et al. (2002) European J. Pharmacol. 438:129-135 used a combination of *in vitro* and *in vivo* testing, very similar to the methods taught in the specification, to identify the MCH antagonist T-226296, a (-) enantiomer of N-[6-(dimethylamino)-methyl]-5,6,7,8-tetrahydro-2-naphthalenyl]-4'-fluoro[1,1'-biphenyl]-4-carboxamide, from a library of chemical compounds. Accordingly, one of ordinary skill in the

art could perform the claimed methods using the knowledge in the art and the guidance provided in the specification.

14. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

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Date Eleftheria Maratos-Flier, M.D.

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June 19th 2002
Date

Eleftheria Maratos-Flier
Eleftheria Maratos-Flier, M.D.